DESTRUCTION OF MICRO-ORGANISMS BY PHYSICAL MEANS

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This paper will start with a few definitions of words so that misunderstandings on that score will be reduced to a minimum. The following definitions are taken from The American Illustrated Medical Dictionary.

STERILIZATION: the act or process of rendering sterile; the process of freeing from all germs.

DISINFECTION: the act or process of destroying pathogenic germs or agents.

DISINFECTANT: an agent that disinfects, chiefly by destroying infective agents (pathogenic organisms) or rendering ferments inactive.

GERMICIDE: an agent that destroys germs.

BACTERIOSTASIS: prevention of the growth of bacteria.

ANTISEPTIC: a substance that will inhibit the growth of microorganisms without necessarily destroying them. - (A bacteriostatic agent.)

It is customary to consider the destruction of bacteria under two headings. Killing by physical agents - drying, heat, light, various radiations, electricity - which is called STERILIZATION, and is distinguished from death by chemical poisons, which is called DISINFECTION. This difference is more apparent than real, since probably in all cases death by physical agents involves a chemical reaction in the microbe, induced by the physical change.

When bacteria are subjected to heat or treated with a chemical disinfectant, the cells do not all die at once. This, of course, is not surprising. One might readily suppose that all the cells would not be alike in their susceptibility, with the majority of individuals succumbing at about the same time, but a number showing greater or lesser resistance to the killing agent. Such is found to be the case when higher organisms are killed. (See graph 1).

With bacteria, however, the order of death is different. It is found that the largest number die in the first time interval and that the number killed in each period is a constant proportion of the number alive at the beginning of that period. (See graph 2). Starting with a suspension of 1,000,000 bacteria per cc. and taking samples every minute it might be found that 900,000 were killed the first minute; then, of the surviving 100,000, 90,000 would be killed in the second minute; of the surviving 10,000 - 9,000 would be killed in the third minute; etc. Death occurs at a constant rate.

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The death curve for bacteria is the same sort of a curve as that which expresses the rate of a monomolecular chemical reaction. Such a reaction is one in which the rate of reaction is determined by the concentration of but one of the reacting molecules. Since this substance is being constantly removed during the reaction, it follows that the greatest amount of substance must be changed at the very beginning of the reaction, that the amount changed in a unit of time must constantly decrease, and that the rate of this decrease is constant.

There is a great similarity between the curves for sterilization of cultures and the curve for the coagulation of proteins in pure solution which leads to the conclusion that the monomolecular character of the death curve may be best explained by assuming that the death of bacteria is due to the effect of the lethal agent upon one particular protein in the organism.

Some bacteria are more susceptible to killing agents than others. In general bacteria may be divided into two classes, the spore-formers and the non-spore-formers. Spores are much more resistant to all killing agents than are vegatative cells. The acidfast Mycobacteria are peculiar. In their resistance to drying and chemicals they resemble spores, but they are little more resistant to heat than bacteria without spores.

Since water is necessary to life, it follows that complete dessication must result in death. There is, however, a wide difference in the susceptibility of micro-organisms to this factor. Spores may remain alive in a dried state for years, some of the delicate pathogens may die in an hour when dried. If dried on glass, bacteria will die much more quickly than if dried in sputum or similar material. The sputum protects the contents of the bacterial cell from drying even though the surface is dry.

Bacteria will remain alive much longer when dried, if they are kept in a vacuum or in an inert gas. So it is quite evident that the death of organisms when dried is due to an oxidation. The rate of death by drying proceeds more slowly at a low temperature.

It has long been known that exposure of bacteria to direct sunlight will kill them. A study of the action of different parts of the spectrum upon bacteria showed that it is mainly the invisible ultra-violet light between 2800 and 2540 Angstrom units which is the most destructive. It has been found that Roentgen rays and radium emanations are also destructive to some organisms.

An apparatus for sterilizing the surface of objects, such as glassware, by ultra-violet light with a wave length of 2550 Angstrom units is now on the market under the trade name of "Sterilamp". It is claimed by the makers that this lamp, when properly placed, will completely sterilize the surface of a glass tumbler in one minute or less. These rays, however, will not penetrate any appreciable depth below the surface of any object; if for instance, a glass tumbler is to be sterilized it is necessary to turn the tumbler so that each surface to be sterilized is presented to the direct rays from the lamp. The rays will not penetrate from the outer to the inner surfaces.

HEAT

The death of bacteria by heat is greatly affected by their moisture content. It requires a higher temperature for a longer time to kill dried bacteria than is the case if they are moist. This is probably due to the fact that death by heat is due to the coagulation of some protein within the cell. It requires a higher temperature to render proteins insoluble when dried than when in solution. It is evident that water enters into the coagulation process.

Dry heat is used in the laboratory almost exclusively to sterilize glassware. Generally a temperature of 160°C. is used. One author states that glass and metal may be sterilized in a dry heat oven by heating to 150°C for one hour; or 180°C for one-half hour; or 200°C for five minutes. A dry heat oven resembles the ordinary baking oven used on kitchen stoves so closely that further description is unnecessary.

The resistance of bacteria to moist heat varies considerably. It is usually expressed as the THERMAL DEATH POINT, which is the temperature necessary to kill bacteria in ten minutes when moist. This value will vary from 45 to about 60°C with non-spore-forming bacteria, and is over 100°C with most spore-formers. To attain the latter temperature with moist heat requires the use of steam under pressure. (See sketch of autoclave). Steam under pressure in the autoclave is efficient not only in sterilizing culture media, but also in sterilizing surgical dressings, and in the commercial processing of canned foods. To render culture media sterile by use of the autoclave it must be exposed to a temperature of 100°C (6 lbs.) for one-half hour; or 121°C (15 lbs.) for fifteen minutes; sometimes 20 pounds for 20 minutes is necessary to kill resistant spores. Water or other liquids, under pressure in the autoclave will vaporize but will not boil even though the temperature is well above the normal boiling point of the water or other liquid. This is due to the fact that the pressure rises as fast in the closed autoclave as the vapor pressure within the heated liquid. Boiling is prevented by the rise of pressure, but vaporizing is not. This fact explains how Durham tubes (tubes of broth containing small inverted vials) are filled. When these vials are dropped into the tubes of broth they are, of course, filled with air which can not escape. But when these tubes are heated in the autoclave they trap vapor driven off from the broth. This vapor displaces the air originally in the tube. When the tube is cooled the entrapped vapor condenses which tends to create a vacuum. This is impossible in an open mouthed tube, instead the tubes fill with liquid and sink. Sometimes a small air bubble will remain in the top of the inverted vial. Since almost all the dissolved air is driven from the broth by the process of autoclaving this bubble will be quickly redissolved, usually twenty-four hours is sufficient.

Pasteurization is used in the preservation of foodstuffs which would be injured by higher temperatures, particularly milk. Two methods are used in the pasteurization of milk, the flash method, in which the milk is heated to 75 - 80°C (167 - 176°F) and immediately cooled again; and the holding process, in which it is heated to 60 to 65°C (140 - 149°F) for thirty minutes.

Boiling will of course almost instantly kill all bacteria except those in the form of spores. It is the usual method for sterilizing surgical instruments and hypodermic syringes.

The use of flowing steam, at atmospheric air pressure, has the same efficiency as boiling. (See sketch of Arnold). It is used in the fractional sterilization of culture media and in the cold pack process of home canning of food. This method of sterilization kills only vegetative cells, so the material being sterilized must be placed in the incubator between heating periods to allow any spores present to develop into vegetative forms which can be killed. The material being sterilized is usually heated for one hour on each of three successive days.

The open flame is a very efficient means to sterilize metal instruments which can be heated to redness without injury, and to destroy contaminated wastes.

FILTERS

There are two general classes of filters in common laboratory use: air-filters, such as the cotton plug in the end of a test tube, and liquid filters, such as the Seitz, Jenkins, Chamberland-Pasteur, Berkefeld and Mandler. (See sketches of filters). Berkefeld filters are made of diatomaceous earth. There are three grades of porosity: V course, (pores 8-12 u) which is used to clear solutions but does not retain all bacteria. N normal (pores 5-7 u) which retain ordinary bacteria. W fine, (pores 3-4 u) which retain all bacteria and some viruses. Mandler filters are made of diatomaceous earth, asbestos and plaster-of-paris. There are three grades: preliminary, regular and fine, corresponding approximately to V, N, and W Berkefelds. Chamberland-Pasteur filters are made of unglazed porcelain. There are nine grades. L_1 corresponds to Berkefeld V, L_3 corresponds to Berkefeld N, and L_{13} corresponds to Berkefeld W.

Filters have two actions. They may remove material from the solution by absorption, for the capillary pores of the filter present an extensive surface. Thus they will remove dyes or proteins from solutions which are forced through them. But their pores are also very fine, and they therefore present a seive action, tending mechanically to remove solid particles in suspension. It is not quite certain to which of these two factors the removal of bacteria from fluids is due. There is thought to be a possible third action in the process of filtration. It is possible that bacteria may be held on the surface of filters by an electrical attraction due to differences in charge between the bacteria and the filter.

Filtration should be as rapid as possible, but a force of more than 35-50 cm. of mercury should not be used because of the possibility of forcing bacteria through the pores.

Gross defects may be detected by immersing the candle in water and attempting to blow air through it. If it seems intact, assemble the filter, sterilize in the autoclave, and filter a liquid to which has been added a broth culture of Serratia marcescens (B. prodigiosus) or some similar organism to give about 100,000,000 organisms per cc. Culture liberal amounts of the filtrate. If no growth appears within 48 hours, the filter may be regarded as intact. The filter must be re-sterilized before further use is made of it.

Filter candles may be cleaned by the following method. Soak in some disinfectant such as cresol, which does not coagulate protein. Scrub the surface with a brush, and force through the filter outward water (or salt sol. if the fluid filtered contained globulin) until clean. Boil $\frac{1}{2}$ hour in 2% sodium carbonate, and then in several changes of distilled water. Force water through

the candle until it is clean and all alkali has been removed.

If clogged with organic material, Chamberland candles and Jenkins filter blocks may be dried in a warm oven, then gradually heated in a furnace to a dull red heat, and slowly cooled. Berkéfeld and Mandler filters often crack if so heated. If speed is necessary it is possible to clean a Jenkins filter block by simply holding it in a Bunsen flame by means of forceps or wire until the block becomes red hot. The heating, however, must be started very gradually to prevent cracking.

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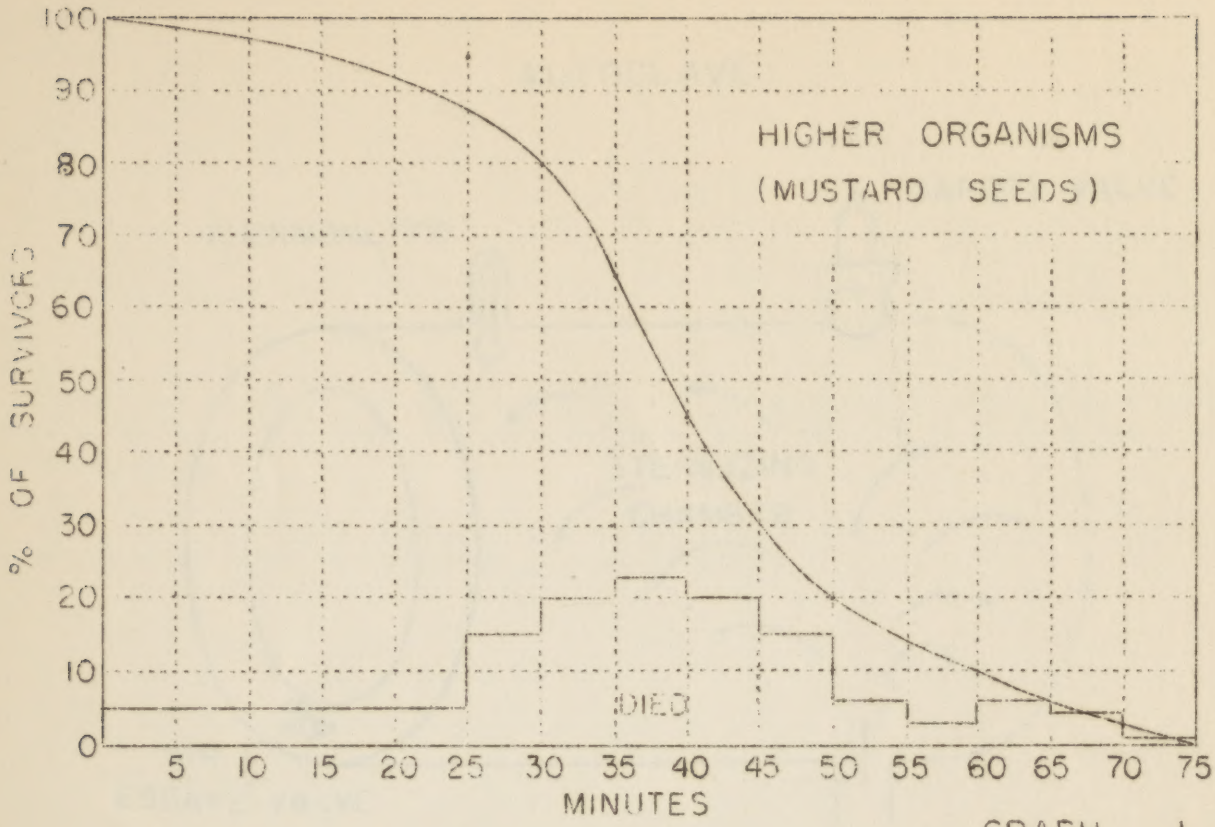
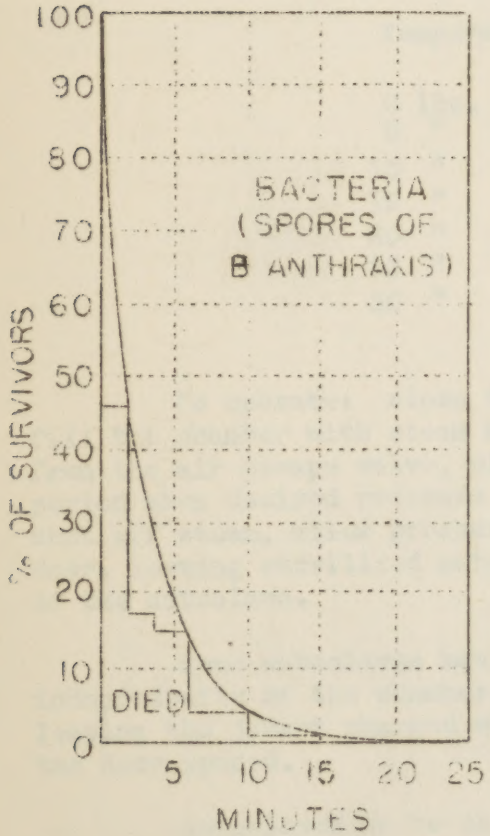
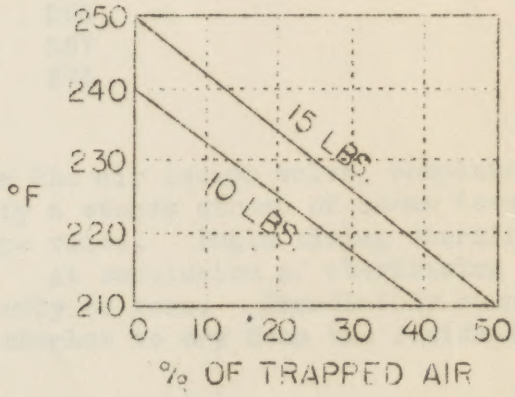


GRAPH 1



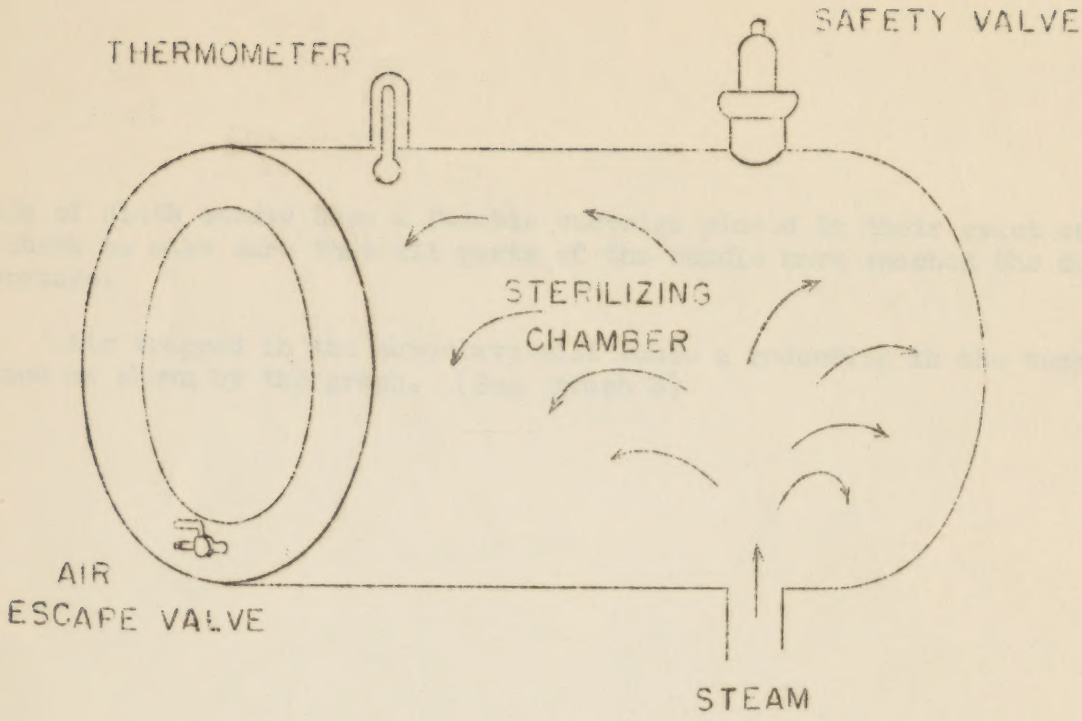
GRAPH 2

TEMP DROP CAUSED
BY AIR IN AUTOCLAVE



GRAPH 3

AUTOCLAVE



Temperature pressure ratio

0 lbs.	100°C	212°F
5 "	108	227
10 "	116	240
15 "	121	250
20 "	127	260
25 "	131	267
30 "	134	274

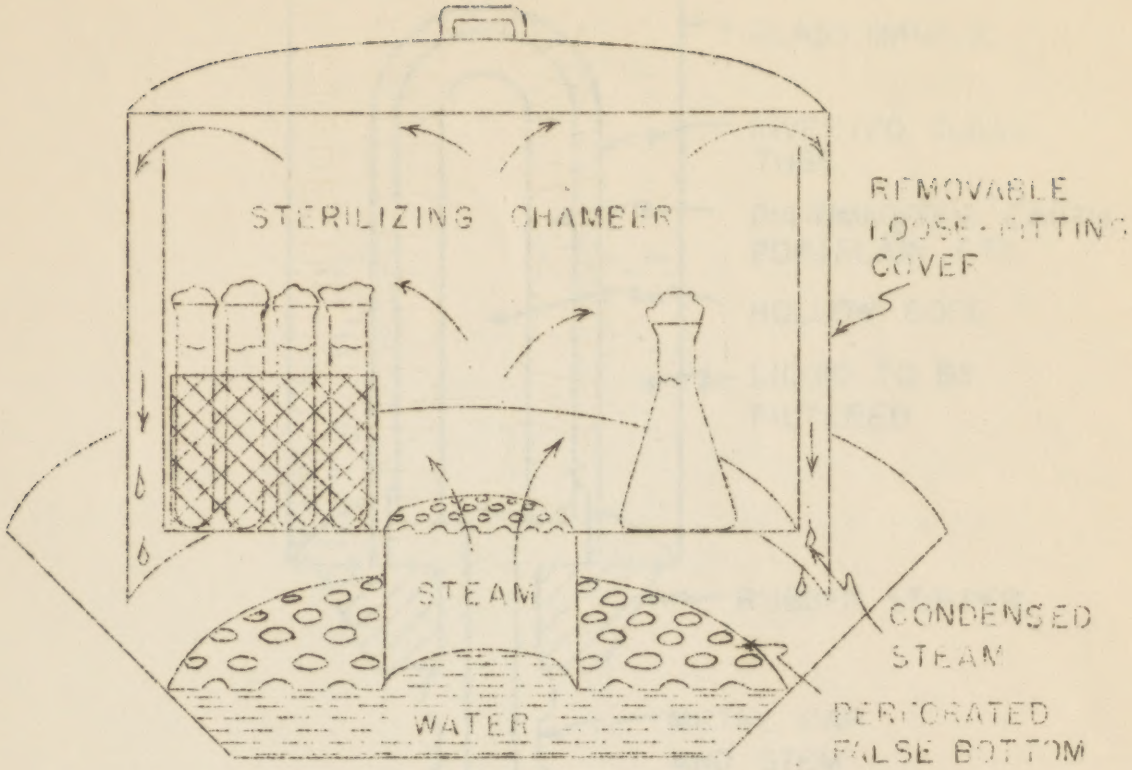
To operate: close the door, open the air escape valve, completely fill the chamber with steam as evidenced by a steady stream of steam issuing from the air escape valve, close the escape valve. Begin timing sterilizing period when desired pressure is obtained. At conclusion of sterilizing period shut off steam, allow pressure to drop slowly to zero. Immediately open the door, leaving sterilized material in the chamber to dry from the residual heat of the autoclave.

Some autoclaves have an inner jacket which can be charged with steam independently of the chamber. In these the drying process can be speeded by leaving the jacket charged when the pressure in the chamber is dropped and the door opened.

Autoclaves to be used for sterilizing large packets of dressings, bundles of sheets, etc., have an arrangement (similar to a water pump) for producing a vacuum in the chamber. In order to secure thorough penetration of bundles of cloth with steam the air must be removed by means of this vacuum pump before the sterilizing process outlined above is started. Large

bundles of cloth should have a fusable cartrige placed in their exact center as a check to make sure that all parts of the bundle have reached the desired temperature.

Air trapped in the autoclave will cause a reduction in the temperature obtained as shown by the graph. (See graph 3)



ARNOLD STERILIZER

This is the ordinary round type. The "Boston Board of Health" or square type is exactly the same in principle. In the square type the outer cover is fixed, entrance to the sterilizing chamber is affected by means of double doors.

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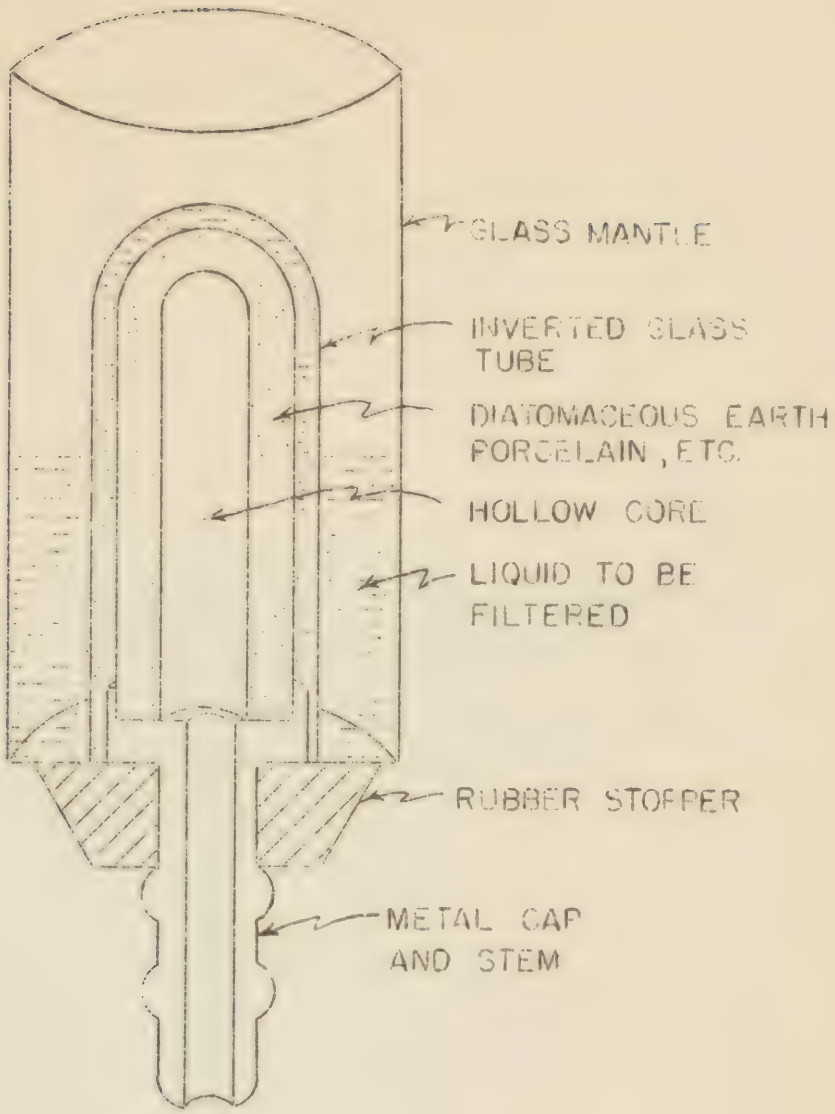
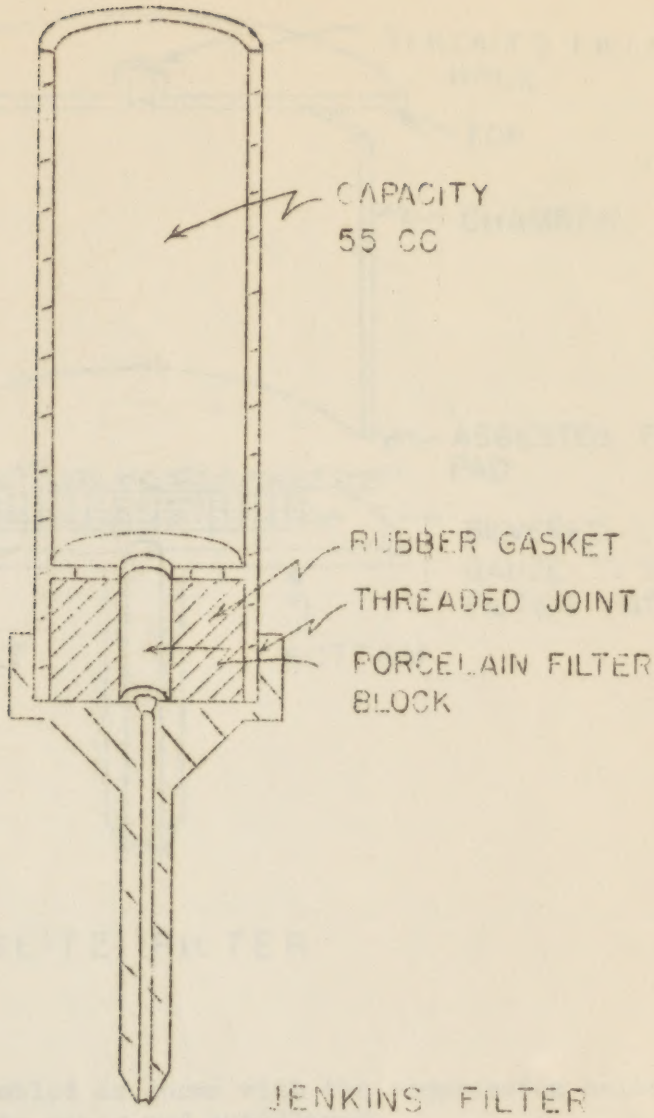


Diagramatic sketch showing construction of Berkefeld, Mandler and Chamberland-Pasteur filters with accessories for the same.

To use, the outfit diagramed above, minus the inverted glass tube, is mounted on a suction flask. The liquid to be filtered is poured into the mantle and the inverted glass tube dropped over the filter candle. The suction is started. The air trapped under the inverted glass tube is drawn through the filter candle creating a vacuum in the inverted tube. This causes the tube to fill with the liquid to be filtered. When all the air is removed a vacuum is created in the filter core and the liquid is drawn through. The liquid continues to pass through the filter until the level of the liquid in the mantle drops sufficiently to allow air to enter the inverted tube.

Liquid will not pass through the filter where any part of the candle is exposed to air because air passes through the filter pores so easily as to destroy the necessary vacuum within the hollow filter core.

The inverted glass tube is a means of reducing loss of filterable liquid. The diagram shows that without the inverted tube the tip of the filter candle would be exposed to air and the filtering process stopped much more quickly, with a consequent greater loss of filterable liquid.

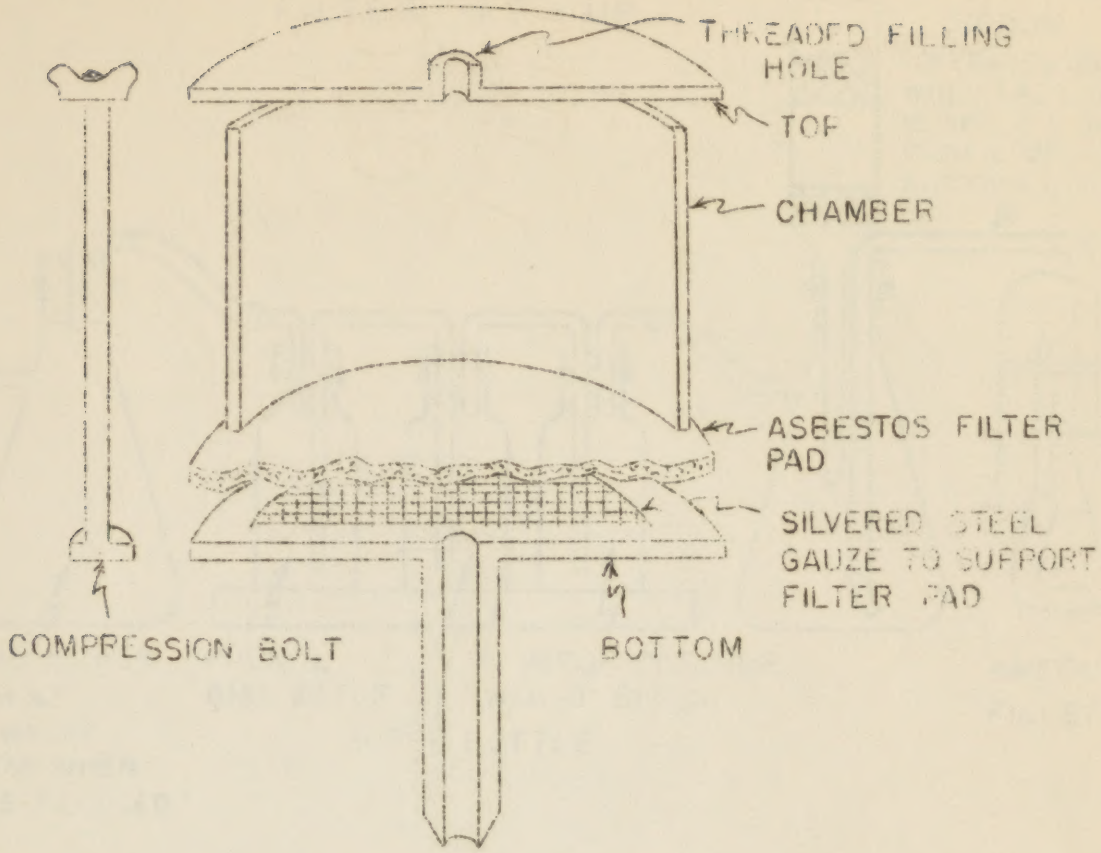


JENKINS FILTER

For small quantities difficult to filter with either Berkefeld or Mandler cylinders since, in the usual mounting, these filters cease to function as soon as the top is exposed to the air.

With the Jenkins filter as much as 0.6 ml can be recovered from a total of 1 ml fluid because only 0.4 ml is absorbed in the pores of the porus porcelain block, which is held tightly by compression between the metallic cylinder and the metal funnel. The whole apparatus can be sterilized in the autoclave, which should not be done with utmost compression on the rubber gasket because rubber is quickly destroyed when sterilized under strong pressure. After removal from the autoclave the filter should be tightened for use.

The porus porcelain block can be cleaned either by burning in the flame or in a furnace, which procedure oxidizes the organic material left in the pores. Because of the metallic construction, there is practically no breakage. The porosity of the porcelain block is indicated by the fact that sterile filtrates are to be had by filtering 24-hour broth cultures of B. prodigiosus, B. typhosus, B. coli and Staphylococcus aureus.



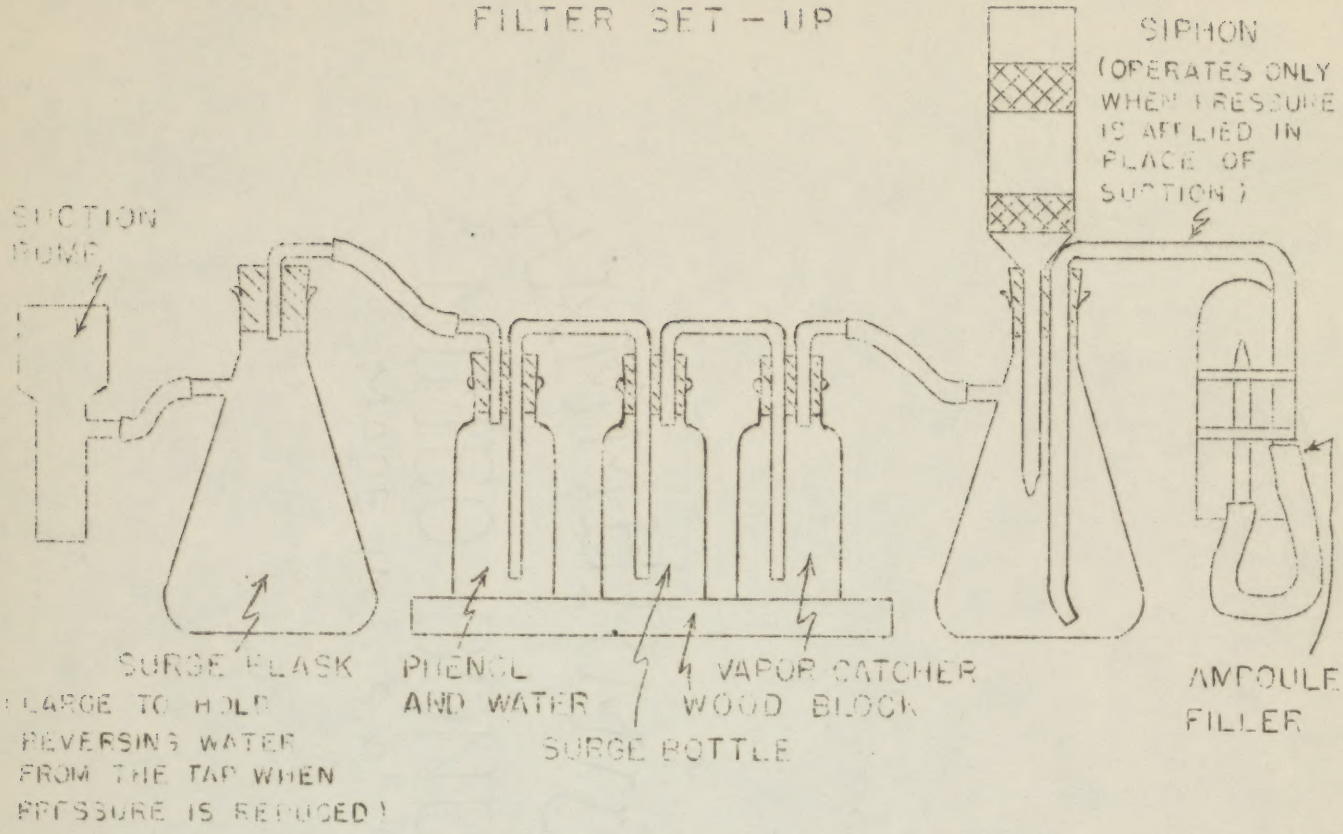
SEITZ FILTER

This filter is assembled as shown with the compression bolts (4) screwed down tight, wrapped in paper and autoclaved.

It is the only filter which will work under pressure (applied through the filling hole) or suction in the usual manner.

The asbestos pads are used only once and discarded. Excellent for preliminary filtration.

FILTER SET - UP



The filter set up diagramed above is an excellent method of preserving a sterile filtrate through possible pressure fluctuations on the water line.

If the suction pump is attached directly to the filter flask a constant water pressure must be maintained to prevent contamination of the filtrate by water surging backward with every drop in pressure. It is not always possible to maintain a constant water pressure. A surge flask is sometimes interposed between the suction pump and the filter flask, but this allows unsterile air to pass into the filter flask with consequent risk of contamination. If the three bottles mounted on a wood block are interposed between the surge and filter flasks, the bottles being sterilized prior to use, unsterile air from the surge flask is at least disinfected before reaching the filter flask, thus greatly reducing the chance of contamination.